

CHARACTERIZATION OF A ASTACIN-LIKE ACTIVITY FROM
HEPATOPANCREAS OF THE SPIDER *NEPHILENGYS CRUENTATA*
(ARACHNIDA)

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Astacin (EC 3.4.24.21) are metalloendopeptidase from Clan MA family M12, which may be involved in digestion and processing of biologically active peptides. This family of enzymes is found throughout the animal kingdom and also in some bacterial species. In order to characterize endopeptidases involved in spider digestion homogenate samples from *Nephilengys cruentata* hepatopancreas as well as elution fractions from anion exchange chromatography and gel filtration were used to assay different proteolytic enzymes using: hemoglobin, casein-FITC, Ac-Ala-Ala-Ala-pNa, Z-FR-MCA as substrates. None activity was observed using Ac-Ala-Ala-Ala-pNa as substrate. *Nephilengys cruentata* hepatopancreas presented cysteine-proteinase activity evidenced by activity on hemoglobin and Z-FR-MCA, dependence on cysteine and EDTA at the reaction medium, inhibition by E-64 and absence of inhibition by PMSF. Besides that, an activity verified using casein-FITC as substrate, which presented anomalous migration on a gel filtration column, a pH optimum of 8.0, a molecular weight of 11 kDa, absence of inhibition by PMSF, E-64 or pepstatin and inhibition by phenantroline and EDTA, indicated the presence of a metalloendopeptidase also involved in spider digestion. Some of these properties and literature data indicate that this activity should be classified as an astacin-like enzyme. Separation on an anion exchange chromatography (Hitrap Q column) has distinguished two activities (Yield: 800% Purification factor: 7x). Other chromatographic strategies are being tested to purify these enzymes. Supported by: FAPESP and CNPq/PIBIC